BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

सत्यमेव जयते

INTELLECTUAL PROPERTY **INDIA**

GOVERNMENT OF INDIA
MINISTRY OF COMMERCE & INDUSTRY,
PATENT OFFICE, DELHI BRANCH,
W - 5, WEST PATEL NAGAR,
NEW DELHI - 110 008.

I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the documents filed in connection with PCT Application as stated below

PCT/IN01/00182 dated 18th October 2001

Witness my hand this 19th day of April 2004.

(S.K. PANGASA)
Assistant Controller of Patents & Designs

HOME COPY

PCT REQUEST

IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

0	For receiving Office use only	
0-1	International Application No.	PCT/IN01/00182
0-2	International Filing Date	ER (18.10.01)
0-3	Name of receiving Office and "PCT International Application"	THE PA... (RO/IN) PCT INT...

0-4	Form - PCT/RO/101 PCT Request	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.03.2001)
0-5	Petition The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	Receiving Office (specified by the applicant)	Indian Patent Office (RO/IN)
0-7	Applicant's or agent's file reference	IN/PA-98
I	Title of invention	CHOLESTEROL LOWERING STRUCTURED LIPIDS WITH OMEGA 6 PUFA
II	Applicant	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
II-5	Address:	an Indian Registered body incorporated under the Registration of Societies Act (Act XXI of 1860) Rafi Marg 110 001 New Delhi India
II-6	State of nationality	IN
II-7	State of residence	IN
II-8	Telephone No.	91-011-6962560
II-9	Facsimile No.	91-011-6968819
II-10	e-mail	ipmd@vsnl.net.in

PCT REQUEST

IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

III-1	Applicant and/or inventor	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	RAO, Reena
III-1-5	Address:	Central Food Technological Research Institute Mysore 570 013 Karnataka India
III-1-6	State of nationality	IN
III-1-7	State of residence	IN
III-2	Applicant and/or inventor	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	SAMBAIAH, Kari
III-2-5	Address:	Central Food Technological Research Institute Mysore 570 013 Karnataka India
III-2-6	State of nationality	IN
III-2-7	State of residence	IN
III-3	Applicant and/or inventor	
III-3-1	This person is:	applicant and inventor
III-3-2	Applicant for	US only
III-3-4	Name (LAST, First)	LOKESH, Belur, Ramaswamy
III-3-5	Address:	Central Food Technological Research Institute Mysore 570 013 Karnataka India
III-3-6	State of nationality	IN
III-3-7	State of residence	IN

PCT REQUEST IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	GABRIEL, DEVADOSS, CALAB
IV-1-2	Address:	KUMARAN & SAGAR 84-C, C6 LANE (OFF CENTRAL AVENUE) SAINIK FARMS 110 062 NEW DELHI India
IV-1-3	Telephone No.	91-011-6533182
IV-1-4	Facsimile No.	91-011-6533889
IV-1-5	e-mail	postmaster@kumaransagar.com
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW MZ SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH&LI CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	**NONE**	
VI	**Priority claim**	**NONE**	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	–	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	1	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	–	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	–	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	–	

PCT REQUEST IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

VIII-2-1	**Declaration: Entitlement to apply for and be granted a patent** Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51bis.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate: Name:	in relation to this international application COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH is entitled to apply for and be granted a patent by virtue of the following:
VIII-2-1 (i)		RAO, Reena of Mysore Karnataka India is the inventor of the subject matter for which protection is sought by way of this international application
VIII-2-1 (i)		RAO, Reena of Mysore Karnataka India is the inventor of the subject matter for which protection is sought by way of this international application
VIII-2-1 (ix)	This declaration is made for the purposes of:	all designations except the designation of the United States of America

PCT REQUEST IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

IX	Check list	number of sheets	electronic file(s) attached
IX-1	Request (including declaration sheets)	6	-
IX-2	Description	10	-
IX-3	Claims	2	-
IX-4	Abstract	1	EZABST00.TXT
IX-5	Drawings	2	-
IX-7	TOTAL	21	
	Accompanying items	paper document(s) attached	electronic file(s) attached
IX-8	Fee calculation sheet	✓	-
IX-17	PCT-EASY diskette	-	Diskette
IX-19	Figure of the drawings which should accompany the abstract	NO	
IX-20	Language of filing of the international application	English	
X-1	Signature of applicant, agent or common representative	Dr. Calab Gabriel	
X-1-1	Name	GABRIEL, DEVADOSS, CALAB [IN/PA-98]	

FOR RECEIVING OFFICE USE ONLY

10-1	Date of actual receipt of the purported international application	18 OCTOBER 2001 (18.10.01)
10-2	Drawings:	
10-2-1	Received	Yes
10-2-2	Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/EP
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT (ANNEX - FEE CALCULATION SHEET) IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

(This sheet is not part of and does not count as a sheet of the international application)

0	For receiving Office use only	
0-1	International Application No.	PCT / IN01/ 00182
0-2	Date stamp of the receiving Office	17 OCTOBER 2001 @ 13:36:00

0-4	Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet	
0-4-1	Prepared using	PCT-EASY Version 2.92 (updated 01.03.2001)
0-9	Applicant's or agent's file reference	IN/PA-98
2	Applicant	COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, et al.

	Calculation of prescribed fees		fee amount/multiplier	total amounts (USD)	total amounts (INR)
12-1	Transmittal fee	T	⇨		0
12-2	Search fee	S	⇨	846	
12-3	International fee				
	Basic fee				
	(first 30 sheets)	b1	382 USD		
12-4	Remaining sheets		0		
12-5	Additional amount	(X)	9 USD		
12-6	Total additional amount	b2	0 USD		
12-7	b1 + b2 =	B	382 USD		
12-8	Designation fees				
	Number of designations contained in international application		90		
12-9	Number of designation fees payable (maximum 6)		6		
12-10	Amount of designation fee	(X)	82 USD		
12-11	Total designation fees	D	492 USD		
12-12	PCT-EASY fee reduction	R	-117 USD		
12-13	Total International fee (B+D-R)	I	⇨	757	
12-17	TOTAL FEES PAYABLE (T+S+I+P)		⇨	1,603	
12-19	Mode of payment		cheque		

VALIDATION LOG AND REMARKS

13-2-4	Validation messages Priority	Green? No priority of an earlier application has been claimed. Please verify
13-2-7	Validation messages Contents	Yellow! The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.

PCT (ANNEX - FEE CALCULATION SHEET) IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

13-2-8	Validation messages Fees	Green? Please confirm that fee schedule utilized is the latest available
13-2-10	Validation messages Annotate	Green? The name of the person signing the request or/and the capacity in which the person signs has/have not been indicated. Please be informed that some receiving Offices require that this information be present along with the signature.

PCT

IN/PA-98

Original (for SUBMISSION) - printed on 17.10.2001 07:36:48 PM

PCT-EASY INFORMATION SHEET

(For applicant use only, DO NOT submit this sheet with the international application)

VALIDATION LOG

Green?	**Priority** No priority of an earlier application has been claimed. Please verify
Yellow!	**Contents** The power of attorney or a copy of the general power of attorney will need to be furnished unless all applicants sign the request form.
Green?	**Fees** Please confirm that fee schedule utilized is the latest available
Green?	**Annotate** The name of the person signing the request or/and the capacity in which the person signs has/have not been indicated. Please be informed that some receiving Offices require that this information be present along with the signature.

Before submitting the International Application, please carefully verify that:

-the information contained on printed Request form is correct;
-Box X of the Request form has been signed;
-all elements of the international application as indicated in Boxes VIII and IX of the Request form have been attached; and,
-the diskette containing the PCT-EASY zip file of the International Application has been enclosed and has been clearly labeled "PCT-EASY", with the applicant's or agent's file reference, and the first applicant's name.

ATTENTION

DO NOT modify any indications on the Request form printout. The electronic version of the PCT-EASY application has been locked. If an error or an omission is discovered at this time, you must reopen the stored form for submission, perform necessary amendments and immediately resubmit the form. Finally, a NEW submission diskette must be created manually by resending the corrected stored form to the diskette. The previously created printout and submission diskette must be destroyed in order to prevent the possibility of erroneously sending it to the RO.

ABSTRACT

Unique structured lipids obtained from interesterifying coconut oil with free fatty acids obtained from hydrolysis of triglycerides of vegetable source, said structured lipids rich in omega 6 polyunsaturated fatty acids and medium chain fatty acids and a process for the production of said structural lipids.

CHOLESTEROL LOWERING STRUCTURED LIPIDS WITH OMEGA 6 PUFA

TECHNICAL FIELD

The present invention relates to cholesterol lowering structured lipids containing omega 6 polyunsaturated fatty acids and a process thereof.

BACKGROUND ART

Coconut oil is a kernel oil which is a natural source of MCFA (53% of C 8:0 – C 12:0). Its lauric acid content is very high (48%). The lauric fats provide high nutritional value because either the portal or lymphatic systems can absorb them. They provide excellent nutrition for critically ill patients and do not cause any undue coronary difficulties despite their saturation. In fact, the lauric fats provide unexpected usefulness in protein catabolism, yielding positive nitrogen balance and enhanced protein formation. But coconut oil contains very low levels of polyunsaturated fatty acids (PUFA) (1.8% of linoleic acid). Long term feeding of coconut oil as the sole source of fat in the diet of experimental rats has shown EFA deficiency symptoms characterized by scaly dermatitis, excessive water loss through the skin, impaired growth and reproduction and poor wound healing. In addition to this, myristic and palmitic acids that contribute to around 33% of the total fatty acids of coconut oil have been shown to be hypercholesterolemic which is a risk factor for cardiovascular disease.

Medium chain fatty acids (MCFA) comprise fatty acids with 6 to 12 carbon chain length. MCFA offer numerous health benefits. They are easily absorbed, transported via the portal system and rapidly metabolized to yield quick energy and is not deposited in the body as fat. Medium chain triglycerides (MCT) have clinical applications in the treatment of fat malabsorption disorders, gall bladder disease, hyperlipidemia, obesity and deficiency of the carnitine system. But MCT alone cannot function as an ideal fat source for humans as they do not provide essential fatty acids (EFA) (EFA cannot be synthesized by the body and must be therefore ingested in the diet).

Linoleic acid is an essential omega 6 PUFA as it cannot be synthesized by mammals. Linoleic acid can be found in seeds of most plants except coconut, cocoa and palm nuts. It is utilized for the synthesis of complex lipids that provide the permeability barrier to the epidermis. They also help to maintain optimum levels of unsaturation in tissue lipids. It has been found to have a reducing effect on plasma cholesterol and an inhibitory effect on arterial thrombus formation. In the body, linoleic acid is metabolized to form arachidonic acid, which is a substrate for eicosanoid biosynthesis.

Structured lipids are triacylglycerols containing mixtures of short-, medium-, and long-chain fatty acids attached to a glycerol backbone for specific functionality. Structured lipids are formed by

(a) hydrolysis and esterification;

(b) interesterification;

(c) lipase-interesterification;

(d) traditional chemical methods or

(e) genetic manipulation.

The structured lipids are particularly useful because of the way in which they are metabolized. Specific fatty acids can be attached to specific portions of the glycerol backbone to ensure that these fatty acids are absorbed in a specific manner in the digestive process.

Enzymatic acidolysis with specific lipase provides an efficient method to improve the nutritional and physical properties of lipids by incorporating the required acyl groups into specific positions of the triacylglycerols.

A physical blend of MCFA rich triacylglycerols and PUFA rich triacylglycerols does not improve the absorption or metabolism of the fatty acids since each of the individual triacylglycerol maintains its original absorption rates.

Safflower oil is a natural source of linoleic acid (68% of total fatty acids). Under controlled reaction conditions employing immobilized sn-1-3 specific microbial lipases, the saturated fatty acid of coconut oil can be partially replaced to incorporate the required amount of omega 6 PUFA from free fatty acids of safflower oil thus obtaining a unique structured lipid rich in MCFA and omega 6 PUFA.

Because of faster absorption, Medium chain triglycerides (MCT) are useful as a calorie source in the treatment of hospitalized patients. Some hospitalized patients, particularly critically ill patients, require total parenteral nutrition and have a high risk of infection. These patients often have difficulty in obtaining the proper amount of nutrients and energy from the diet; a diet that both minimizes the risk of infection and provides quick nutrition would be of vast benefit to these patients. These diets must provide the essential fatty acids, including a limited amount of specific omega.6 fatty acids

It has been theorized that a structured lipid containing a medium chain fatty (C.6 -C.12) acid residue may provide improved absorption of other fatty acids attached to the structured lipid. A recent paper by Jensen (Am. J. Clin. Nutr.1992, 60: 518-524) disclosed that a structured lipid containing medium chain fatty acid residues and long

chain fatty acid residues (omega 3 fatty acids from fish oil) are absorbed faster by the body than the physical mixture of the same fatty acids. There is no suggestion or teaching that a specific structured lipid containing MCFA and omega 6 PUFA would be useful to modify the lipid profile of the body.

Reference may be made to an article, Kaimal, and Saroja, (1989, Journal of Oil Technologist's Association of India, Jan- Mar. 2-10). Wherein coconut oil was modified by enzymatic transesterification with safflower oil. The drawbacks of this method are that the products could not be expected to have specific type of fatty acid composition as two types of triacylglycerols were involved and the incorporation was poor as only preliminary studies were carried out.

Reference may also be made to the article by Shimada, Y., Sugihara, A., Maruyama, K., Nagao, T., Nakayama, S., Nakano H., and Tominaga, Y., (1996) J. Am. Oil Chem. Soc.73, 1415-1420, wherein safflower oil or linseed oil were subjected to enzymatic acidolysis to produce structured lipids containing both EFA and MCFA. But the draw back of this invention is that the MCFA was a highly purified one obtained commercially which is not economical.

Similarly, reference may also be made to the article by Akoh C.C.,.Jennings B.H and. Lillard. D.A, (1996) J. Am. Oil Chem. Soc. 72, 1059-1062 who used EPA ethyl esters (omega 3 PUFA) to modify evening primrose oil which is a rich source of γ-linolenic acid, an omega 6 PUFA. The drawbacks of this invention were that, the structured lipid developed in this case had very low levels of MCFA and serves only as a single rich source of both omega 3 and omega 6 PUFA rather than aiming at optimal nutrition. Moreover, the EPA ethyl esters used are from commercial sources.

Reference may be made to Lee, K-T and Akoh C.C., (J.Food Biochem. 1999, 23.197-208) wherein structured lipids were synthesized from synthetic tricaprylin and fish oil free fatty acids rich in omega 3 PUFA. The product when fed to mice for 21 days showed lower levels of serum total cholesterol, LDL cholesterol and triacylglycerol in comparison with soybean oil. However it is not very clear from this study whether a physical mix (blending) of tricaprylin and fish oil (having same fatty acid composition as the structured lipids) would have the same effect as the structured lipids on the serum lipid profile. The soybean oil used as a control in this study does not provide a good comparison with the structured lipid, which had a different fatty acid composition.

U.S. Pat. No. 5,661,180 to DeMichele, et al., describes a structured lipid, which

provides substantial benefits in terms of modifying the prostanoid synthesis pathway, resulting in an improved response to endotoxic shock and other stress states. This structured lipid includes three components formed on a glycerol backbone. The first component is either alpha-linolenic acid or dihomogamma-linolenic acid. The second component is a medium chain (C 6 – C 12) fatty acid residue and the third component is a C 18 – C 22 fatty acid residue. The structured lipids in this case are prepared from a physical blend of oils subjected to a transesterification reaction to yield a reaction product that contains the structured lipids of this invention. The draw back of this is that the fatty acids will be randomized among the triglycerides of the two oils selected and there will not be any specificity in the positioning of fatty acids in the structured lipids.

Canadian Patent Application 2000391 with a WPI Accession Number of 90-139962/19 discloses the triglyceride 2-(alpha-linolenoyl)/gamma-linolenoyl)-1,3-di (octanoyol/decanoyl) glycerol as useful in nutritional lipids. It is suggested that these triglycerides are useful as components in nutritional compositions. The fatty acids are essential for control of tonus of smooth-muscle cells in the blood vessels or the tonus of the smooth muscle cells in the lungs and thus are useful in the control of respiratory distress. This reference neither suggests nor discloses the specific structured lipids of this invention nor the methods of using them.

European Patent Application Number 87114297.2 discloses a triglyceride having a C.8 to C. 14 fatty acid residue at the 2-position of the triglyceride and a residue of C. 18 or higher fatty acids at the 1 and 3 positions thereof. There is no suggestion or disclosure of the specific structured lipids of the invention or the benefits that can be realized by feeding the structured lipids of this invention.

US Patent No. 5,962,712 deals with structured lipid containing gamma-linolenic or dihomogamma-linolenic fatty acid residue, a medium chain (C6-C12) fatty acid residue and N-3 fatty acid residue.

DISCLOSURE OF THE INVENTION

The main object of the present invention is to provide a process for the synthesis of unique structured lipids containing omega 6 polyunsaturated fatty acids using natural sources.

Yet another object of the present invention is to provide a process for the synthesis of structured lipids that are rich in MCFA and omega 6 PUFA, which is nutritionally advantageous by way of being hypocholesterolemic and hypotriglyceridemic.

Still, another object of the present invention is to provide a process for the synthesis of structured lipids containing omega 6 polyunsaturated fatty acids that could be clinically administered to patients in parenteral nutrition.

Further another object of the present invention is to provide a process of Enzymatic acidolysis to produce fats (structured lipids) with a better triglyceride-distribution than known natural fats.

Yet, another object of the present invention is to provide a process for structured lipids with an improved melting behaviour as they will hardly contain any trisaturated triglycerides.

Yet another object of the present invention is to provide a process to develop a product, for use in a controlled diet for critically ill patients, comprising lauric acid to provide quick energy and n-6 PUFA to modulate their eicosanoid production especially in immune compromised patients and linoleic acid to take care of EFA requirement.

Yet another object of the present invention is to develop a product by modifying coconut oil with the incorporation of omega 6 PUFA, which has a lower melting point than natural coconut oil.

The present invention also provides a process for the manufacture cholesterol lowering structured lipids from coconut oil.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, the present invention provides a process for the synthesis of cholesterol lowering structured lipids from coconut oil containing omega 6 polyunsaturated fatty acids .

An embodiment of the present invention, wherein hydrolysis of triglycerides derived from an vegetable oil source, is done by a known method to obtain free fatty acids, rich in omega 6 PUFA.

In another embodiment of the present invention, wherein interesterification of vegetable oil with free fatty acids thus obtained, is done at 1:3 molar ratio and at a temperature range of 37- 55°C, for a period of 6-48 hours, using a solvent, an hydrocarbon selected from petroleum ether, dioxane, isooctane, n- hexane, toluene etc.,.

In yet another embodiment of the present invention, wherein the reaction is controlled by employing immobilized sn-1-3 lipase for enzymatic acidolysis thereby incorporating the required acyl groups into the specific positions of the triacylglycerols.

In still another embodiment of the present invention, purification of reaction products is conducted using adsorption chromatography along with solvents selected from hexane and diethyl ether at a ratio ranging from 85:5 to 95:5 to obtain structured lipids.

Further, in yet another embodiment of the present invention, structured lipids are recovered following scale up in the range of 88-92% having cholesterol-lowering capacity in the range of 10-36%

Yet another embodiment of the present invention, wherein the fatty acid is derived from a vegetable source of safflower oil.

Still another embodiment of the present invention, wherein the vegetable oil is derived from coconut oil.

Another embodiment of the present invention, wherein the interesterification is carried out using lipase enzyme at 5%(w/w) of the substrates.

Yet another embodiment of the present invention, wherein *Rhizomucor meihei* an immobilized lipase is used.

Still another embodiment of the present invention, wherein an immobilized lipase *Rhizomucor meihei* that can be utilized up to 25 cycles without loss of activity, is used, thus ensuring economic viability.

Further another embodiment of the present invention, wherein the natural source of triglycerides is from coconut oil and fatty acid is derived from safflower oil for acidolysis reaction.

The present invention also provides for a unique structured lipids rich in MCFA (Medium Chain Fatty Acid) and n-6 PUFA (Polyunsaturated fatty acid), which is nutritionally beneficial in being hypocholesterolemic and hypotriglyceridemic.

An embodiment of the present invention, provides for structured lipids comprising lauric acid that produces quick energy for critically ill patients.

Yet another embodiment of the present invention, wherein the structured lipids comprise n-6 PUFA to modulate eicosanoid production in immune compromised patients.

Still another embodiment of the present invention, provides structured lipids that possess a very low melting point 12-15^{0}C and remains as a liquid without phase separation

Yet another embodiment of the present invention, provides structured lipids comprising a cod liver oil fatty acids and triaglycerols of coconut oil for optimal nutrition.

Still another embodiment of the present invention, comprises structured lipids having n-6 PUFA levels from 1.8 % in the unmodified coconut oil to 45.5% in the structured lipid.

Yet another embodiment of the present invention, wherein the structured lipids comprise purity of triglycerides up to 96mg.

In still another embodiment of the present invention, wherein structured lipids are recovered following scale up in the range of 88-92% having cholesterol lowering capacity in the range of 10- 36%.

BRIEF DESCRIPTION OF THE ACCOMPANIED DIAGRAMS

Fig 1 is a pictorial representation of the steps involved in the synthesis of structured lipids.

Fig 2 is a Graphical Diagram depicting the lower melting point of the Structured lipids enriched in Omega 6 PUFA.

The following examples are given by way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

Example 1

Reaction conditions were optimized for the production of structured lipids from coconut oil with safflower oil free fatty acids employing a statistical design (Response surface methodology). Under optimized conditions as predicted by the model for maximum incorporation, 100mg of coconut oil and 132 mg of free fatty acids from safflower oil were taken and 0.5 ml of hexane was added (the volume was maintained through out the reaction). The reaction mixture was taken in a 25 ml conical flask.11.5 mg of immobilized lipase from *Rhizomucor meihei* was used and the incubation was carried out in an orbitally shaking waterbath at 160 rpm at 39°C for 48.5 hours. The modified triglycerides (structured lipids) was separated by preparative thin layer chromatography with petroleum ether: ethyl ether: acetic acid (80:20:1 v/v/v) as the developing solvent. The analysis of fatty acids by gas chromatography showed a maximum increase in omega 6 PUFA levels from 1.8% in the unmodified coconut oil to 45.5% in the structured lipids. The recovery of structured lipids was 96mg.

Fatty Acid Composition (mol%) of Unmodified Coconut Oil and Free Fatty Acids Obtained from Safflower Oil and Structured Lipid.

Fatty acids	Coconut Oil	Safflower Oil Free Fatty Acids	Structured Lipid
Caprylic(8:0)	2	-	-
Capric(10:0)	3	-	-
Lauric(12:0)	48	-	17
Myristic(14:0)	24	1	11
Palmitic(16:0)	9	8	9
Stearic(18:0)	3	3	2
Oleic (18:1)	9	20	15
Linoleic (18:2) (omega 6)	2	68	46

Example 2

Enzymatic acidolysis reactions were scaled up in lab scale batch reactions of 100g triglycerides and 132g of free fatty acids from safflower oil taken in a 2 liter Erlenmeyer flask that served as the bioreactor. 11.5g of immobilized lipase was used and the reaction was carried out in 500 ml of hexane. Incubation was carried out for 48.5 hours at 39°C in an orbitally shaking waterbath.

Triglycerides were separated from the reaction mixture by column chromatography. A mixture of 20g each of alumina and silica gel (100-200 mesh ize) were activated at 200°C for 2 hrs and cooled in a desiccator. Slurry of this was made in hexane and packed in 4cm x 35cm glass columns.

30g of the sample from the reaction mixture was loaded on the column and eluted with 350 ml of hexane: diethyl ether (95:5 v/v). The fractions containing triglycerides were pooled and solvent was removed in a vacuum rotary evaporator. The purity of triglycerides was checked by thin layer chromatography. Recovery of the scaled up product was 90g for structured lipids rich in omega 6.

The reactions when carried out at optimized conditions showed 46% incorporation of omega 6 PUFA into coconut oil.

Example 3

The structured lipids were fed to rats in the diet as the sole source of fat at 10% levels for a period of 60 days. The serum and liver cholesterol was decreased by 10 and 36% respectively and the triglyceride level reduced by 17 and 16% respectively in the serum and liver.

Lipid Profile of Rats fed Coconut oil, Blends and Structured lipid

Dietary Groups	Total Cholesterol		Triglycerides	
	Serum (mg/dl)	Liver (mg/g)	Serum (mg/dl)	Liver (mg/g)
Coconut oil	74.54 ± 1.3	7.21 ± 0.2	169.90 ± 6.9	12.8 ± 2.2
Coconut oil: Safflower oil (1:0.7) Blend	74.76 ± 0.7	7.80 ± 1.0	169.5 ± 6.7	9.07 ± 0.9
Structured lipid (omega 6 PUFA rich)	66.84 ± 1.4	4.62 ± 0.3	140 ± 4.8	6.93 ± 0.7

Example 4

The structured lipids were subjected to DSC studies wherein their peak melting temperature and solid fat content was determined. The peak melting temperature of structured lipids enriched in omega 6 fatty acids was 12.7°C while that of coconut oil was 20.8°C .

Advantages:

(1) The present invention uses fatty acids from natural sources to create novel structured lipids with potential impacts on nutrition and health

(2) The process could be employed for the modification of coconut oil, which is deficient in EFA by effectively incorporating omega 6 fatty acids.

(3) The production could be scaled up without loosing the efficacy of the process.

(4) The use of natural sources of triglycerides and fatty acids for the acidolysis reaction and the use of immobilized lipase which could be reused up to 25 cycles without loss of activity ensures that the process is economically viable.

(5) Nutritionally the structured lipid proved to be more advantageous than oil blends with similar fatty acid composition or unmodified coconut oil in being hypocholesterolemic and hypotriglyceridemic.

(6) The structured lipid, rich in MCFA and omega 6 PUFA, will provide a lipid source, primarily for use in a controlled diet for critically ill patients, which has lauric acid to provide quick energy and the changes in eicosanoid synthesis seen with omega 6 PUFA feeding will improve immunocompetence and a reduced inflammatory response to injury. Patients in need of elemental diets will benefit from having their immunocompetence improved.

(7) The structured lipid obtained as a result of enzymatic acidolysis reactions displayed an improved melting behavior compared to coconut oil or the oil blends. The structured lipid has a lower melting temperature than unmodified coconut oil thus maintaining them in the liquid state even at low temperatures.. Hence the structured lipid at a temperature of 12 - 15°C remains a liquid without phase separation.

CLAIMS:

1. Unique structured lipids obtained from interesterfying coconut oil with free fatty acids obtained from hydrolysis of triglycerides of a vegetable source, said structured lipids contain up to 46mol % of omega 6 polyunsaturated fatty acids and rich in medium chain fatty acids.
2. Unique structured lipids as claimed in claim 1, wherein the structured lipids comprise lauric acid that produces quick energy for critically ill patients.
3. Unique structured lipids as claimed in claim 1, wherein the structured lipids are rich in MCFA (Medium Chain Fatty Acid) and n-6 PUFA (Polyunsaturated fatty acid), which is nutritionally beneficial in being hypocholesterolemic and hypotriglyceridemic
4. A unique structured lipid as claimed in claim 1, wherein the structured lipids having cholesterol-lowering capacity in the range of 10- 36%.
5. A unique structured lipid as claimed in claim1, wherein the recovery of scale up of structured lipids is in the range of 88- 92%
6. Unique structured lipids as claimed in claim 1, wherein the structured lipids comprise n-6 PUFA to modulate eicosanoid production in immune compromised patients.
7. A unique structured lipid as claimed in claim 1, wherein the structured lipids are having a very low melting point 12-15^{0}C that remains as a liquid without phase separation
8. A unique structured lipids as claimed in claim 1, wherein the structured lipids are having a safflower oil fatty acids and triaglycerols of coconut oil for optimal nutrition.
9. A unique structured lipid as claimed in claim 1, wherein the structured lipids comprise n-6 PUFA levels from 1.8% in the unmodified coconut oil to 45.5% in the structured lipids.
10. A unique structured lipids as claimed in claim 1, wherein the serum and cholesterol lowering capacity of the lipids in mammals is 10% and 36% respectively.
11. A process for production of cholesterol lowering structured lipids from cod liver oil rich in omega 6 polyunsaturated fatty acids (omega 6 PUFA), said process comprising;

(a) hydrolyzing triglycerides of vegetable oil source by known method to obtain free fatty acids rich in omega 6 PUFA;

(b) interesterifying coconut oil with the free fatty acids obtained from step(a) at a preferable molar ratio of 1:3 molar ratio;

(c) incubating with immobilized immobilized sn-1-3 lipase at a temperature range of 37-55^{0}C for a period of 6-48 hours using a solvent for enzymatic acidolysis thereby incorporating the required acyl groups into specific positions of the triacylglycerols;

(d) separating the reaction products using adsorption chromatography using solvents selected from ethers, hexane and optionally with 1 part of acetic acid to obtain the structured lipids; and

(e) recovering the structured lipids by scaling up in the range of 88-92%.

12. A process as claimed in claim 11, wherein the triglycerides are selected from a natural sources namely coconut oil.

13. A process as claimed in claim 11, wherein the fatty acids are selected from a vegetable source of safflower oil.

14. A process as claimed in claim 11, wherein the ethers are selected from group comprising petroleum ether, diethyl ether.

15. A process as claimed in claim 11, the solvent is selected from petroleum ether, dioxane, isooctane, n- hexane, toluene.

16. A process as claimed in claim11, wherein the ratio of ethers:hexane used is the range of 85:5 to 95:5.

17. A process as claimed in claim 11, wherein the interesterification is carried out using lipase enzyme at 5-10%(w/w) of the substrates.

18. A process as claimed in claim 11, wherein the immobilized lipase is obtained using *Rhizomucor meihei.*

19. A process as claimed in claim 11, wherein an immobilized lipase obtained from *Rhizomucor meihei* can be used up to 25 cycles without loss of activity, thus ensuring economic viability.

ABSTRACT

Unique structured lipids obtained from interesterifying coconut oil with free fatty acids obtained from hydrolysis of triglycerides of vegetable source, said structured lipids rich in omega 6 polyunsaturated fatty acids and medium chain fatty acids and a process for the production of said structural lipids.

FIGURE 1

SYNTHESIS OF STRUCTURED LIPIDS

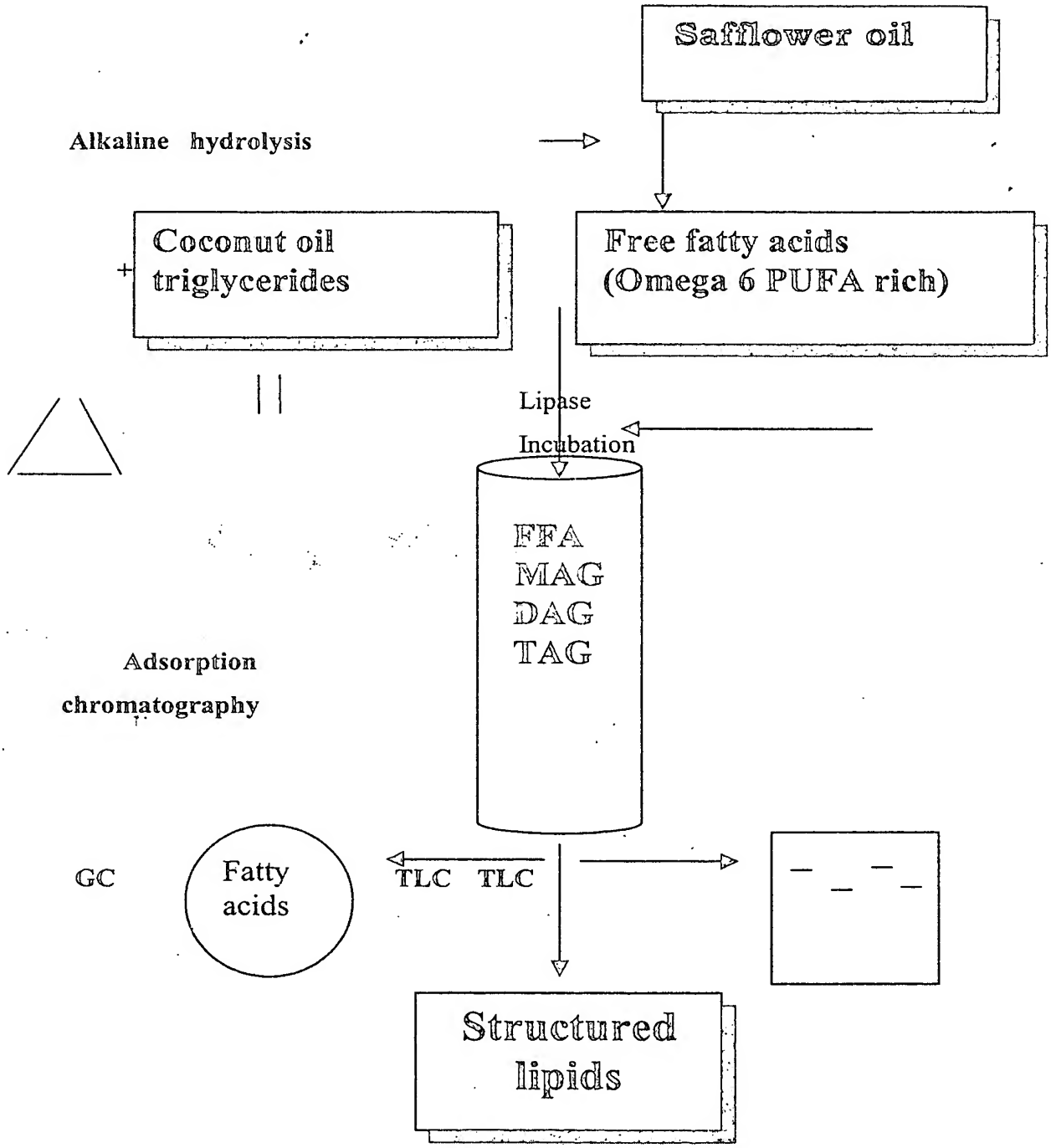
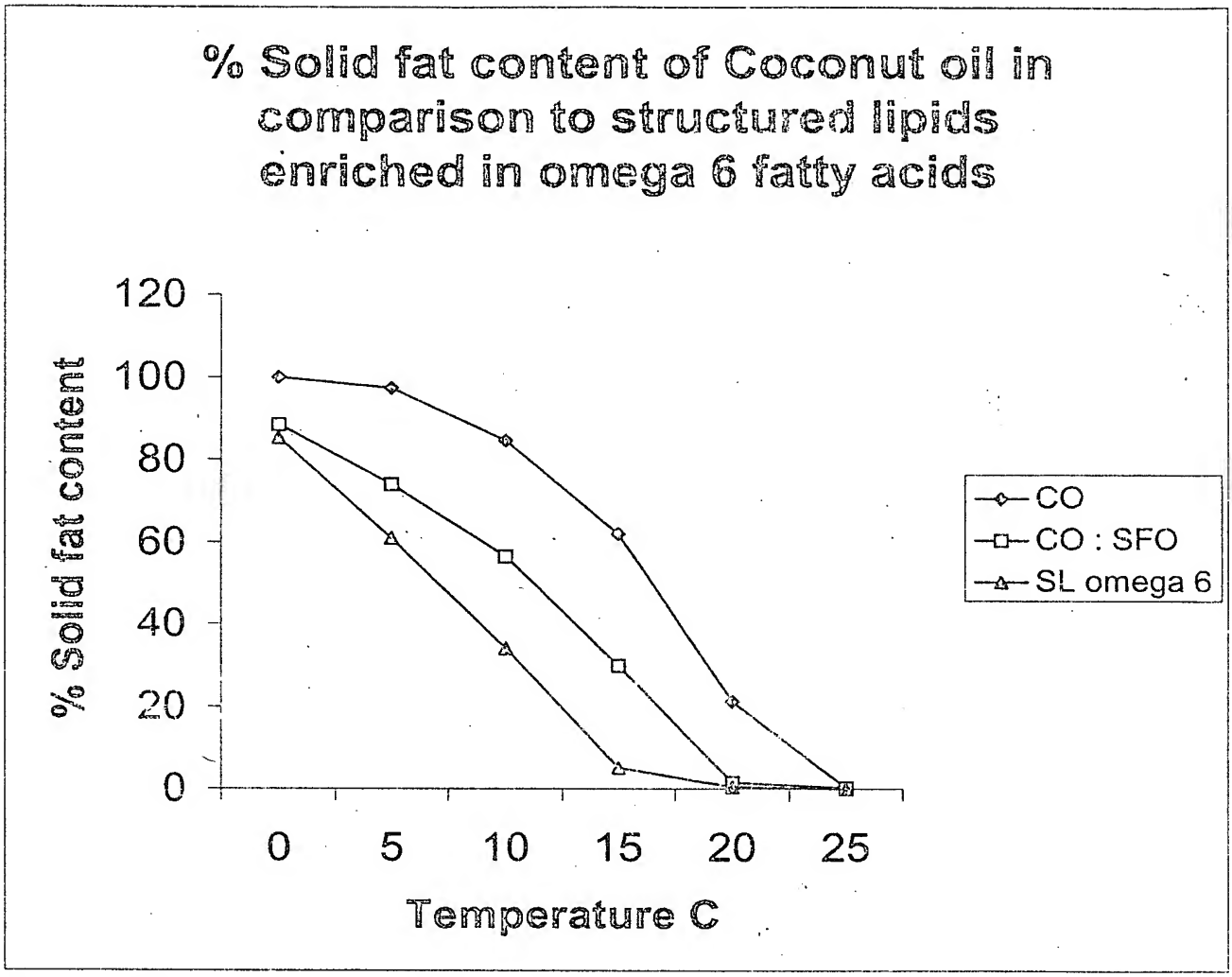


FIGURE 2



CO- Coconut oil

CO: SFO – coconut oil: safflower oil blended at 1: 0.7 molar ratio

SL omega 6- Structured lipids enriched in omega 6 PUFA